Journal of Forestry Research (2013) 24(3): 547–552
DOI 10.1007/s11676-013-0386-1

ORIGINAL PAPER

# Ultrastructure of stridulating organ of *Xylotrechus rusticus* L. (Coleoptera, Cerambycidae) and behavioral responses to alarm sounds

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Received: 2012-10-20; Accepted: 2013-01-23


**Abstract:** We used scanning electron microscope (SEM) to observe the ultrastructure of stridulating organs in *Xylotrechus rusticus* L. We compared the morphological structure and size of stridulating organs, the numbers of a tooth-like part used in stridulation and its presence in females and males of this beetle. The alarm sound of *X. rusticus* was recorded first when it was stimulated, then we tested behavioral responses to this alarm sound. The alarm sound of *X. rusticus* has a warning effect on conspecifics.

**Keywords:** *Xylotrechus rusticus* L.; stridulating organ; behavioral response to sound

## Introduction

As an effective way of communication among insects, sound is prevalent in many insect species (Cai 1988; Cao and Cheng 2004). The initial research on stridulating organs of insects was undertaken mainly to describe the vocal structure of the chirping insects such as species of Orthoptera (Shi and Zheng 1998; Shi et al. 1999; Yin 1984; Lin and Shao 1982; Lin 1985; Lin 1987) and other groups of insects. Alexander et al. (1963) studied the sound and behavior of 40 beetle species of 8 families. By using the scanning electron microscope, researchers have studied the ultrastructure of the stridulating organs of the chirping insects of the superfamily Acridoidea (Wang et al. 1988; Shi et al. 2000; Xie and Zheng 2001; Xie and Zheng 2005). By analyzing sound characteristics and phylogenetic analysis based on the mitochondrial cytochrome b gene fragment, Ronara et al. (2010) studied the mixed species diversity of ants under natural conditions in the tropical rainforest. Sound is an effective way to identify species of ants. In a review of auditory communication of moths, Williame (1999) considered that the evolution of the tympanic membrane through which moths detect sound was related to the predation pressure from insectivorous bats that echolocate. Evolution of the tympanic membrane enabled moths to identify position or location, and contributed to mating. Through analyses of signal communications between small herbivorous insects such as stinkbug, Andrej and Meta (2003) concluded that herbivorous insects produce vibration signals by friction or vibration of their body parts. Moreover, the characteristic low frequency signal has a relatively narrow frequency band peak mainly in the 100 Hz range, its frequency can be adjusted to form advanced harmony. Ryo et al. (2008) also found that moths can generate low quiet ultrasonic courtship signals by friction between special scales.

Most Cerambycidae insects generate sound by friction with their chest sounder. Presence or absence of stridulating organs has been used as one criterion for distinguishing species of the subfamily in Cerambycidae, but there is no description of the differences in morphology between species (Shao 1996; Xin et al. 1985). There are brief descriptions of ultrastructural stridulating organs of the longhorn beetle (Zhang et al. 1997; Luo et al. 2000).

Miller (1971) recorded friction sound waveforms from three species of beetles and considered that the "nod" movement of beetles is related to the worm ventilation and the ventilation. Cheng (1991) recorded sound waves and observed the behaviors of 15 beetle species, each of which produced friction sounds with identifiable and unique characteristics. Cheng (1993) showed that adult *Anoplophora horsfieldi* (Hope) had two different sounding methods, one by chest friction sound and the other by elytra vibration. Chest friction sound could be continuous to warn predators and had intraspecific communication biological significance. Elytra vibration sound, which was intermittently generated, could be induced by external stimulation and was related to predator resistance.

*Xylotrechus rusticus* L.（Cerambycidae）is one of the main branch-borers in northeast China. We observed the ulstrastructure of chest stridulating organs in this beetle by

The online version is available at http://www.springerlink.com

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scanning electron microscope (SEM). We recorded the signal sound waves and observed and recorded beetle behavior. Our objective was to develop a foundation for further research on the relationship between the sound and behavioral responses in this beetle and explore the role of sound in behaviors such as interspecific communication, host location, and courtship mating. Our research goal was to provide a reference for exploring new methods to control the damage caused by this beetle in forests.

## Materials and methods

We undertook two separate investigations. First was a study of body parts of captive *X. rusticus*. to describe its sound generating apparatus. This involved SEM examination and photography. Second was a laboratory study of live, adult *X. rusticus*. to document their behavioral responses to playback of sounds recorded from free-ranging *X. rusticus*.

### Specimen sources

All of the specimens for ultrastructure study were taken from the insect specimen room in the School of Life Sciences, Northeast Forest University, Harbin, China. Live larvae of *X. rusticus* for the study of behavioral response to sound were collected from timbers of *Populus nigra* L. in Kuandian County, Daqing City in October 2010. The larvae were reared in timber sections in the laboratory until they became adults.

### Sample preparation for description of sound-generating apparatus

Pronota and mesonota were removed from male and female beetles and immersed in 20% sodium hydroxide for 24h, attached muscle tissues were removed and samples were first cleaned with sterile water and finally cleaned using ultrasonic cleaner for 5 min before dehydration in serial ethanol at concentrations of 60%, 80% and 100%, for 2 min each.

The samples of stridulating organs were fixed in the sample stage with conductive adhesive tape. We sprayed gold on samples by use of an ion sputtering instrument, and then observed and photographed samples under SEM (FEI-QUANAT-200) (Cheng 2006).

### Behavioral response to playback of recorded sounds

Samples of live adults (reared from larval stage) were divided into three groups, 60 samples for each group. Single samples of the first group were placed in a 5 cm diameter plastic insect box. For the second group, five samples of the same gender were placed in one plastic insect box (6 cm × 13 cm × 20 cm). And for the third group, five samples from the same gender were placed in a windtunnel box (100 cm × 200 cm).

Recorded alarm sounds of male *X. rusticus* were played at 70-75 db as a stimulus signal and recorded human applause sound at 70 db was played as the control. Sounds were broadcast by an HP computer speaker for 2 s at intervals of 30 s. The stimulation was repeated 10 times. Behavioral responses of *X. rusticus* L. to the broadcast sounds were recorded within 30 s after stimulation. Beetles remaining stationary or moving in the opposite direction from the sound source were marked as positive reactions (predator avoidance), while samples showing irregular action or toward the direction of the sound source were marked as negative reactions (no predator avoidance).

Alarm sounds of live (captive beetles recorded in lab), adult *X. rusticus* were recorded around 10 o'clock in the morning on a Sony ICD-SX900 recorder placed within 5 cm of calling beetles (Cheng 1993). The recorded sounds were edited by Windows Movie Maker 2.6 software, and sound intensity was measured using an HS6288 Decibel Meter (Red Sound Instrument Factory, Jiangxi).

We divided the behavioral responses to playback of recorded alarm sounds into three classes. Class I: beetles rapidly crawled in response to playback of sounds. Class II: due to the size of the testing box, beetles displayed different behavioral responses. Class III: beetles did not move or crawl after hearing the playback of recorded alarm sounds.

### Data Processing

Differences in behavioral responses between adult female and male *X. rusticus* were compared with Tukey Multiple analysis. Data variance and multiple comparisons were analyzed with statistical software SPSS 17.0. Paired T tests were used to detect differences in bioassay data ($\alpha = 0.05$) (Liu et al. 2005; Hern & Dorn 2004; Tooker et al. 2005).

## Results and analysis

### Ultrastructure of the stridulating organ of *X. rusticus*

The stridulating organ of *X. rusticus* is located on the middle of the mesonotum, anterior to the elytra and covered by pronotum (Fig. 1). The stridulating file (plank) and plectrum (friction brush or files or bristles) of the stridulating organ could be found under light microscope after removing the cover of pronotum (Fig. 2).

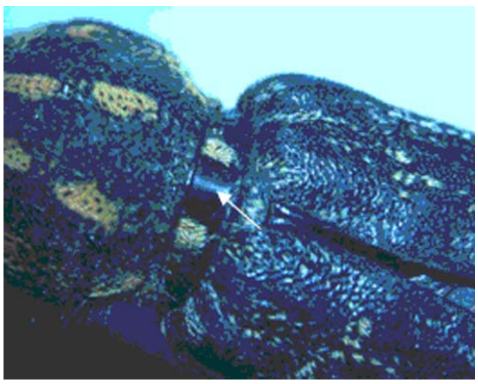

**Fig. 1 The location of plank of female *X rusticus* (×10) (arrow instruct)**

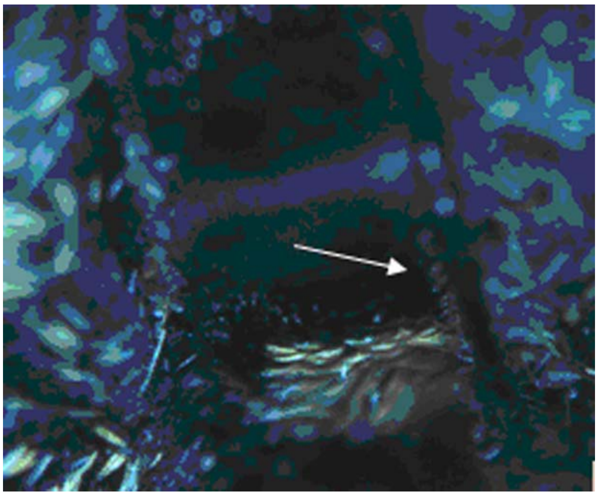

**Fig. 2 The stridulatory brush of female *X. rusticus* (×25) (arrow instruct)**

The stridulating organ of *X. rusticus* was composed of the stridulating file and plectrum. No vertical lines were found in the female or male stridulating file, which was oval-shaped in the mesonotum under SEM observation (Fig. 3a and Fig. 3b).

The transverse diameter of the plank in females was 522.2±77.9 µm and the vertical diameter was 1,056.1±71.3 µm (Fig. 3a). The stridulating file was composed of the pronunciation trabecula (teeth) arranged evenly in the middle with teeth numbering 21±1 rows per 100 µm (Fig. 3c). The edge of the pronunciation trabecula was thin and some teeth were irregular due to bifurcation. The friction brush was a row of hollow bristles growing at the front of the posterior margin of the pronotum with average friction brush length of 96.9±9.6 µm and average file diameter of 7.2±1.4 µm (Fig. 3e).

The plank of males was slightly smaller than for females, consistent with the shorter body length of males than females. The transverse diameter of the plank in males was 502.5±41.6 µm and the vertical diameter was 1,024.6±224.0 µm (Fig. 3b). The teeth were arranged evenly in the middle of the male plank and were more dense than in females. The number of teeth in males was 23±1 rows per 100 µm. Some pronunciation trabecula was irregular due to bifurcation (Fig. 3d). As in females, the friction brush in males was also a row of hollow bristles growing at the front of the posterior margin of the pronotum with average friction brush length of 90.4±15.8 µm and average file diameter of 5.8±0.8 µm (Fig. 3f).

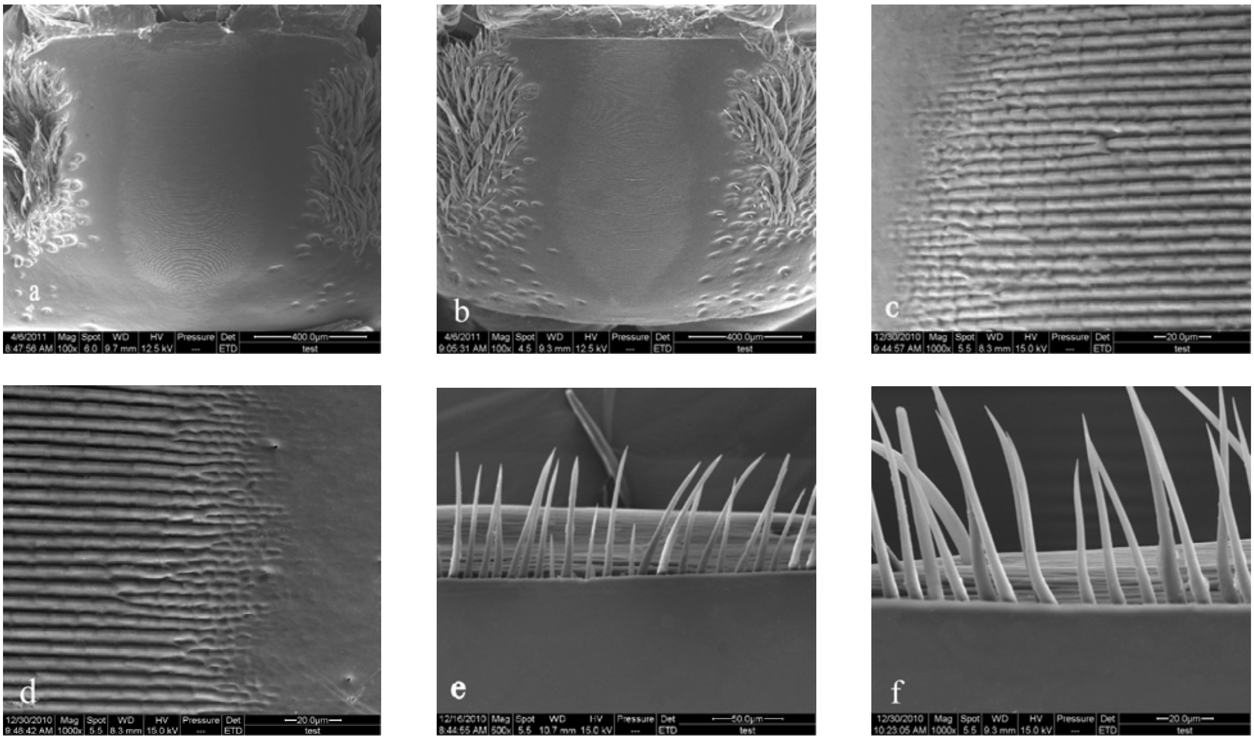


**Fig. 3 Stridulatory organs of *X. rusticus* L.（Note: a, c, e belong to female; b, d, f belong to male）**

Manner of pronunciation of *X. rusticus* L.

Cheng (1991) reported that the stridulating organ in longhorn beetle is composed of the oval plank in the mesothorax and posterior margin bursting striate, or plectrum, in the pronotum. This conclusion did not mention the bristles growing regularly at the posterior margin of the pronotum. Cheng (1993) reported that in nature, the prothoracic shield is covered by the posterior margin in the pronotum of *Anoplophora horsfieldi* (Hope).

Transverse embossment of the prothoracic shield is connected with the teeth. When the beetle displays the “nod” movement, its head and prothorax along the mesothorax joints raise and drop rapidly, causing the transverse embossment along the surface of

the stridulating zone to move back and forth vertically to produce the sound by contact with the teeth. Reports to date show only the stridulating zone but not the bumping process. We used SEM to observe the stridulating file and posterior margin bursting striate of the pronotum and identified rows of regular bristles at the posterior margin of the pronotum. At the onset of sound generation, these bristles connected with the stridulating file while the beetle displayed the "rising" and "nod" movements. When we placed these rows of bristles into a 20% sodium hydroxide solution for 24 hours, they remained rigid without distortion and could contact the surface. These special bristles are arranged regularly and densely, however, the common feeling bristles were irregular and sparse. So the bristles (friction brush) at the posterior margin of the pronotum play a major role in rubbing the stridulating file, enabling the beetle to produce sound.

Alarm sound of the *X. rusticus*

When the adult longhorn beetle was stimulated by external conditions, the stridulating organ could produce alarm sound by rapid rubbing. When stimulated by constriction, eight (80%) male and seven (70%) female beetles produced sound similar to "zhi zhizhi" in apparent alarm and defense against the disturbance. In nature, when predators disturb the beetle, it produces the alarm sound in defense against predation (Cheng 1991).

The intensity of alarm sound changes with body form: larger beetles produce louder sound, while the sound produced by smaller beetles is undetectable by the human ear (Cheng 1993). Beetles of intermediate body size in our study produced sound of moderate intensity. Three males and one female (20% of beetles in our study) produced sound clearly detected by the human ear. If the stimuli were weak, the beetles could produce sound or not. If the stimuli were strong, the beetles could produce continuous sound. Production of sound stopped when the stimulus was terminated. According to the classification standards of Cheng (1991), the sensitivity of *X. rusticus* to external stimulus is moderate.

Behavioral responses of *X. rusticus* L. to the alarm sound

After playing the alarm sound, beetles in different states of activity generally reduced movement levels. After replaying the alarm sound three times, more than 90% of beetles did not move in response. After replaying five times, no beetles moved but in a separate test group, two male beetles feigned death, an extreme reaction to the alarm sound. In the third group with larger activity space, all beetles rapidly moved in the direction opposite to the sound source. These responses showed that the alarm sound had a warning significance to the beetles and elicited alert or escape behaviors.

Behavioral responses of the three tested groups of beetles showed that a significant reaction compared to control beetles ($p$ = 0.000). Responses of male and female beetles did not differ ($p$ = 0.901) (Tables 1 and 2).

The behavioral reaction rate to the alarm sound was 93.33% for males and 95% for females. The overall mean individual reaction rate was 94.17% (Table 2). Auditory communication signals were generally received by the beetles in test populations. The alarm sounds generated by *X. rusticus* had a warning significance to its population members who displayed avoidance or escape behaviors.

**Table 1. The behavioral response of *X. rusticus* to its male alarm voice**

| Group | | positive reaction (♀) | negative reaction (♀) | no reaction (♀) | reaction rate (♀) | positive reaction (♂) | negative reaction (♂) | no reaction (♂) | reaction rate (♂) |
|---|---|---|---|---|---|---|---|---|---|
| I | adult | 54 | 0 | 6 | 90.00 | 56 | 0 | 4 | 93.33 |
| | contrast | 2 | 3 | 55 | 8.33 | 1 | 3 | 56 | 6.67 |
| II | adult | 57 | 1 | 2 | 96.67 | 53 | 2 | 5 | 91.66 |
| | contrast | 3 | 4 | 53 | 11.67 | 5 | 4 | 51 | 15.00 |
| III | adult | 59 | 0 | 1 | 98.33 | 57 | 0 | 3 | 95.00 |
| | contrast | 5 | 4 | 51 | 15.00 | 4 | 6 | 50 | 16.67 |

**Table 2. Bbehavioral responses of female and male *X. rusticus* to male alarm calls**

| Group | positive reaction | negative reaction | no reaction | Sig.2-tailed | reaction rate |
|---|---|---|---|---|---|
| Female (♀) | 170 | 1 | 9 | 0.001** | 95.00% |
| Male (♂) | 166 | 2 | 12 | 0.000** | 93.33% |

**Note**: "**": $p$ <0.01

## Discussion

Structure of the stridulating organ of *X. rusticus*

Cheng (1991) reported that the ultrastructure of the stridulating organ of longhorn beetle is composed of an oval plank on the mesonotum and the margin of the pronotum. We found no description of the bristles on the margin of the pronotum. The mechanism of sound generation in longhorn beetles was thought to be the movement of the pronotum margin on the oval plank of the mesonotum but this was not supported by photographic records. We used SEM to identify bristles (the friction brush) at the posterior margin of the pronotum of *X. rusticus*. We

hypothesized that the bristles rub the stridulating file first when the beetle generates sound and thus the bristles play a major role in beetle sound generation.

Assessment of behavioral reaction of *X. rusticus* to alarm sound

In nature, the stripe on the surface of the beetle body provides protective coloration that presumably contributes to predator avoidance. Beetles that remain motionless are more difficult to locate. This might explain why beetles did not move at the moment they first heard the alarm sound. This behavior is a predator avoidance strategy. Beetles remained motionless or crawled in the direction opposite to the sound source to avoid predators.

## Conclusion

We have found that the stridulating organ of *X. rusticus* composed of the sound plank (stridulating file) and plectrum (friction brush or files or bristles). The stridulating file was located at the middle of the mesotergum and was covered by the pronotum in its natural state. The sound plank was composed of the pronunciation trabecula (teeth), which were arranged evenly in the middle of plank. The friction brush was a row of hollow bristles growing in the front of posterior margin of the pronotum. The bristles (friction brush) in the posterior margin of the pronotum play a major role in rubbing the trabecula of the beetles, which generated sound.

*X. rusticus* belonged to the moderate stirred species. When it was touches, 80% male and 70% female might produce sound as “Zhi Zhizhi”, which means that the beetle felt fear and showed the resistance to the disturbance. Those male or female *X. rusticus* heard those sound shown staying still or trying to escape from the sound source reaction. There was no significant difference at the reactive intensity or reactive ration between male and female individuals.

The discovery of the ultrastructure stridulating organ, the mechanism of producing alarm sounds and the response of male and femal adult to the playback of the recorded alarm sounds could be used in the in the control of the population of *X. rusticus* L. in the poplar stands. So this study has found a possible way to decrease the damage of this pest on poplar trees.

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